

IN THE CLAIMS:

1. (Twice amended) An isolated microbial cell comprising an Environmentally Limited Viability System, wherein the cell is viable in a permissive environment and non-viable in a non-permissive environment, the system comprising

E1 (a) an essential gene, wherein expression of the gene in the cell is essential to the viability of the cell, and the essential gene is expressed when the cell is in the permissive environment and is not expressed when the cell is in the non-permissive environment, and wherein the essential gene is a copy of a native gene; and

(b) a lethal gene, wherein expression of the gene is lethal to the cell and the lethal gene is expressed when the cell is in the non-permissive environment but not when the cell is in the permissive environment,

wherein the [essential gene corresponds to an inactivated native gene of]native gene is inactivated in the cell.

E2 23. (Twice amended) The cell of claim 1 for use as a vaccine, wherein the cell is viable when in [the]an animal and non-viable when outside of the animal, the essential gene is expressed when the cell is in the animal and is not expressed when the cell is outside of the animal, and the lethal gene is expressed when the cell is outside of the animal and is not expressed when the cell is in the animal, wherein the permissive environment comprises a temperature of about 37°C and the non-permissive environment comprises a temperature of less than about 30°C, wherein the cell is a member of *Enterobacteriaceae*.

27. (Twice amended) A method of making a cell strain with environmentally limited viability comprising stably introducing into a cell

E3 (a) an essential gene, wherein expression of the gene in the cell is essential to the viability of the cell, and the essential gene is expressed when the cell is in the permissive environment and is not expressed when the cell is in the non-permissive environment, and wherein the essential gene is a copy of a native gene;

(b) a lethal gene, wherein expression of the gene is lethal to the cell and the lethal gene is expressed when the cell is in the non-permissive environment but not when the cell is in the permissive environment,

wherein the cell strain is viable in a permissive environment and non-viable in a non-permissive environment,

wherein the [essential gene corresponds to an inactivated native gene of]native gene is inactivated in the cell.

30. (Thrice amended) A method of inducing immunoprotection in a warm-blooded animal comprising
administering to the animal a vaccine comprising a microbial cell comprising an Environmentally Limited Viability System, wherein the cell is viable when in the animal and non-viable when outside of the animal, the system comprising

E4 (a) an essential gene, wherein expression of the gene in the cell is essential to the viability of the cell, and the essential gene is expressed when the cell is in the animal and is not expressed when the cell is outside of the animal, and wherein the essential gene is a copy of a native gene; and

(b) a lethal gene, wherein expression of the gene is lethal to the cell and the lethal gene is expressed when the cell is outside of the animal but not when the cell is in the animal,

wherein [the essential gene corresponds to an inactivated native gene of]the native gene is inactivated in the cell,

wherein the cell is a member of the *Enterobacteriaceae*.

41. (Amended) The cell of claim 1 wherein the essential gene is selected from the group consisting of a gene essential for metabolism of the cell, [or] and a gene essential for growth of the cell.

E5 42. (Amended) The cell of claim 1 wherein the essential gene is a gene essential for cell wall or cell membrane integrity.

43. (Amended) The cell of claim 1 wherein the essential gene is selected from the group consisting of a modification methylase gene, a gene required for nucleic acid replication, or a gene encoding an enzyme that catalyzes steps in the biosynthesis of DAP.

REMARKS

Claims 1-16, 20-32, 33-38, and 41-45 remain pending in the application. Claims 5-9, 15, 21, 22, 25, 26, 36, and 38 are withdrawn from consideration; claims 1-4, 8-14, 16, 20, 23, 24, 27-32, 35, 37, and 41-44 stand rejected. Claims 1, 23, 27, 30, 41, and 42 have been amended to more particularly point out and distinctly claim the invention.

Support for the claim amendments is found at least at page 10, lines 6-10.

Applicants gratefully acknowledge the withdrawal of: (1) the rejection of claims 1-4, 8, 10-14, 16, 20, 23, 24, 27-29, 30-32, 35 and 37 under 102(e); (2) the rejection of claims 3, 23, 24, 29-32 and 35